ELabELN | Template Use Case White Paper

Sanger Sequencing Experiment Template

Structured Documentation for Construct Verification — From Primer Selection Through Alignment-Confirmed Mutation Calling

APPLIES TO

- Academic and life sciences research laboratories
- Biotechnology and molecular biology R&D groups
- Diagnostics and clinical R&D laboratories
- Contract research organizations (CROs)

RELEVANT COMPLIANCE & INTEGRITY FRAMEWORKS

- FDA 21 CFR Part 11 — Electronic records and signatures
- EU Annex 11 — Computerized systems in regulated environments
- GAMP 5 — Software validation for regulated industries
- NIH / NSF reproducibility & clone-provenance documentation for publications and patents

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1. Executive Summary

Sanger sequencing remains the reference method for verifying a defined stretch of DNA with single-base accuracy. When a lab needs to confirm that a clone carries the intended mutation, that a construct assembled correctly, that a CRISPR edit landed where it should, or that a PCR product is the sequence it claims to be, Sanger — not high-throughput sequencing — is the tool of record. It is also a workflow with a long, error-prone paper trail: a primer, a template prep, a dilution, a submission to an outside service, a returned AB1 trace, a quality assessment, an alignment to a reference, and a final mutation call. When that chain is scattered across a submission portal, an email, a spreadsheet, and a notebook, the result is the most common Sanger failure mode: a confirmed clone whose provenance cannot be reconstructed when a reviewer, a patent examiner, or a collaborator asks for it.

The ELabELN Sanger Sequencing Experiment Template solves this by providing a pre-built, fully structured experiment record that loads from the template library in a single click. It ships with a 10-step submission-and-analysis protocol, validated primer references, pre-wired AB1 quality fields, and typed inputs for template, primer, service provider, read direction, read length, Q30, trim positions, and mutation calls. Plasmid stocks, primers, and service-provider records link to the ELabELN inventory, and the tamper-evident audit trail captures every submission, every AB1 download, and every mutation call.

This white paper describes what the template contains, the verification use case it is designed to support, the upstream and downstream workflows it connects to, the benefits it delivers to the scientist, and how a laboratory can deploy it and begin submitting within days.

> **KEY BENEFIT**
> Labs that adopt the Sanger template stop re-keying submission details into provider portals and spreadsheets, gain a queryable mutation-call history across every construct, and produce a clone-provenance record — submission, AB1 trace, and final call in one audit-ready place — that stands up to publication, patent, and 21 CFR Part 11 review.

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2. What Is Sanger Sequencing, and Why Does Documentation Matter?

2.1 The Technique

Sanger (chain-termination) sequencing reads the order of bases in a single DNA template by extending a primer with a mix of normal and fluorescently labelled chain-terminating nucleotides, separating the products by capillary electrophoresis, and reading the resulting four-channel chromatogram — the AB1 trace. A single read typically resolves 700–900 high-quality bases from one primer with the per-base accuracy that makes Sanger the gold standard for confirming a defined region.

This accuracy over a defined window is exactly what construct verification needs. Researchers reach for Sanger to confirm a site-directed mutation, verify a cloned insert, genotype a CRISPR-edited clone, identify a microbial isolate, or confirm a PCR product. Unlike next-generation sequencing — which reads millions of short fragments in parallel for quantitative or genome-scale work — Sanger answers a focused question: does this clone read exactly as intended across this region?

Because most labs outsource the capillary run to a commercial service (Eurofins, GENEWIZ/Azenta, Macrogen) or share an in-house ABI instrument, the workflow spans systems the lab does not fully control. **Capturing the submission metadata, the returned trace, and the final call in one place is what makes a Sanger result reproducible and defensible.**

2.2 The Documentation Problem

Recording Sanger work across a provider portal, an inbox, and a notebook creates a set of interconnected risks:

- Submission details — template, primer, concentration, read direction, chemistry, provider — are re-keyed into a portal and never captured in the lab's own record.
- The returned AB1 trace lives in a download folder or email, disconnected from the experiment that produced it.
- Quality metrics (Q30, trim positions, secondary peaks) and the final mutation call sit in free text, where they cannot be queried or compared across submissions.
- There is no link between the sequencing record and the upstream cloning or mutagenesis experiment that created the construct.
- There is no timestamped, attributed record of who submitted what and when, or who made the final call — a baseline requirement for clone provenance and regulated work.
- Prior submissions for a construct cannot be retrieved without manually searching portals and notebooks, so the mutagenesis trail is lost.

These are the gaps that undermine clone provenance, and they are the gaps this template was designed to close.

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3. Template Overview: What Ships with the Template

The ELabELN Sanger Sequencing Experiment Template is a pre-populated experiment record available to all ELabELN subscribers directly from the template library. It ships configured for verification of a site-directed A206K monomerizing mutation in a pUC19-GFP construct, using outsourced BigDye chemistry from a commercial service provider, with a 10-step protocol covering primer selection through alignment-confirmed mutation calling. The default configuration assumes a standard plasmid prep (200 ng/µL miniprep DNA), a single forward primer (pUC19-fwd), submission for BigDye Terminator v3.1 chemistry on an ABI 3730xl with a 50 cm capillary, and AB1 trace analysis in SnapGene or SeqMan Pro. Every section is editable — the configuration is a tested baseline, not a locked format.

3.1 Template Sections at a Glance

The template populates the existing ELabELN experiment sections your lab already works in. Your team edits a working baseline instead of rebuilding the submission record for every batch.

Section	What it pre-populates
Main Text	Submission objective, primer rationale, and the expected mutation call, authored in the TinyMCE rich-text editor and linked to the upstream cloning experiment.
Extra Fields	Eleven structured, typed fields grouped as Submission, Quality, and Mutation Calling — template, primer, service provider, read direction, read length, Q30, trim positions, intended vs observed mutation, secondary peaks, and alignment status. Typed values are queryable across submissions.
Steps	A 10-step workflow checklist covering primer pick through alignment-confirmed call. Each tick is timestamped to the audit trail; the AB1 download is auto-logged.
Compounds	Sequencing primer, template plasmid, and submission reagents pre-linked from the compound database, with primer designs and template lot carried in automatically.
Links & Resources	Pre-wired to the lab's Sanger Submission SOP v1.4, the pUC19-fwd primer database entry, and the pUC19-GFP-A206K reference sequence map.
Storage & Files	−20 °C plasmid stock storage, an AB1 trace archive, and aligned-reference attachments — AB1 and PDF ready on save.

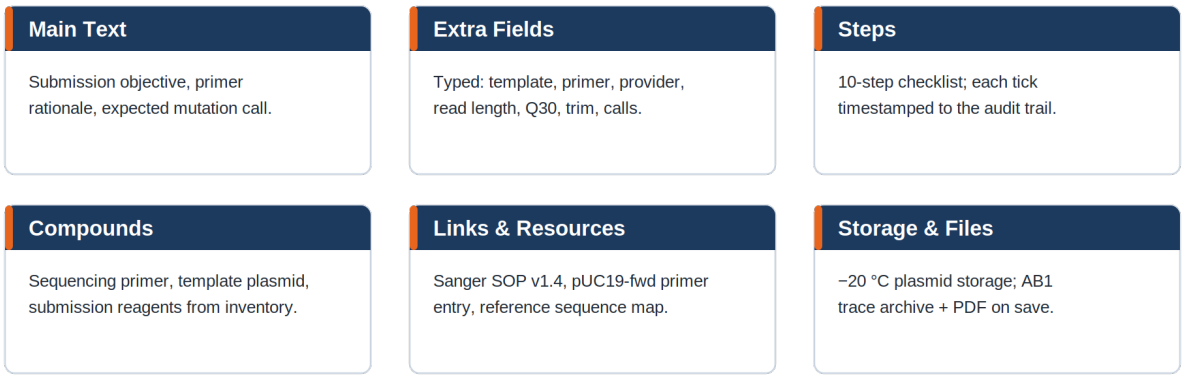
Main Text
Submission objective, primer rationale, expected mutation call.
Extra Fields
Typed: template, primer, provider, read length, Q30, trim, calls.
Steps
10-step checklist; each tick timestamped to the audit trail.
Compounds
Sequencing primer, template plasmid, submission reagents from inventory.
Links & Resources
Sanger SOP v1.4, pUC19-fwd primer entry, reference sequence map.
Storage & Files
−20 °C plasmid storage; AB1 trace archive + PDF on save.

Figure 1. The six ELabELN experiment sections pre-populated by the Sanger sequencing template.

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3.2 The 10-Step Protocol

The workflow checklist is the operational spine of the template. Each step maps to a discrete action and writes a timestamped, user-attributed entry to the audit trail when checked off. Three steps — submission, the BigDye run, and receipt of the AB1 trace — happen off-site at the sequencing service provider, and the template records that hand-off explicitly.

Step	Action	What it captures
1	Pick primer	Primer link and read direction
2	Prep template	Miniprep; template / plasmid link
3	Quantify	Sample concentration (e.g. 200 ng/µL)
4	Dilute & plate	Primer concentration; submission identity
5	Submit (provider)	Service provider; submission ID
6	BigDye sequencing	BigDye v3.1 on ABI 3730xl (off-site)
7	Receive AB1	AB1 trace download auto-logged
8	QC / Q30 trim	Q30 percentage; trim positions
9	Align to reference	Alignment status (SnapGene / SeqMan)
10	Call & sign-off	Intended vs observed mutation; e-signature

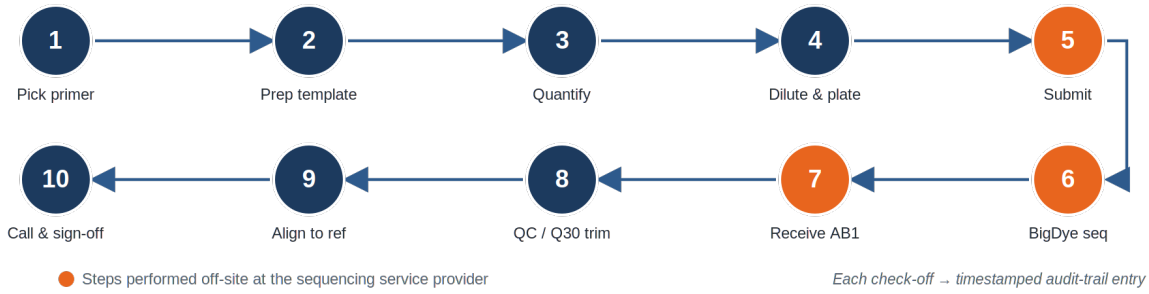

Figure 2. The 10-step Sanger workflow — orange steps run off-site at the service provider; every check-off writes a timestamped entry to the ELabELN audit trail.

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4. The Use Case: When and How the Template Is Used

The template is intended as a tested baseline for academic, biotechnology, and diagnostics research labs that verify DNA constructs. It is configured around a recognizable reference — confirming an A206K monomerizing mutation in pUC19-GFP — but the scenarios it serves are broad.

4.1 Construct and Mutagenesis Verification (Primary Use Case)

The core scenario: a researcher has introduced a site-directed mutation or assembled a construct and must confirm the sequence before using the clone. The template's typed Mutation Calling fields (intended mutation, observed mutation, secondary peaks, alignment status), combined with the linked reference map, capture the verification outcome in a queryable, signed-off record rather than a portal screenshot.

4.2 CRISPR Clone Genotyping

After editing, a lab Sanger-sequences individual clones to determine genotype. The template documents which primer read which clone, the trace quality, and the called edit — and links back to the editing experiment — so the genotype of each clone is traceable and comparable across the screen.

4.3 Microbial Strain Identification

Labs identifying isolates by 16S or marker-gene sequencing use the same submission-and-analysis structure: swap the template type and primer, record the read and the reference match, and retain the trace alongside the call.

4.4 PCR Product Confirmation

When a PCR amplicon must be confirmed, the template's template-type field accommodates a PCR product directly, capturing the read, quality, and identity in the same structured form used for plasmid work.

4.5 Clone Provenance, Multi-PI, and CRO Settings

Because the typed mutation calls and AB1 attachments are retained and queryable, a lab can pull every Sanger submission for a construct and reconstruct the full mutagenesis trail — the clone-provenance record publications and patent applications increasingly require. Every ELabELN edition includes unlimited users, so the PI, postdocs, students, and visiting collaborators all work from the same template; granular permissions let project leads control who can edit submissions versus only review them, which is the basis a CRO needs to standardize documentation across client studies.

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5. Dependencies and Connected Workflows

The Sanger template does not exist in isolation. It sits inside a network of connected workflows — upstream, parallel, off-site, and downstream — that give the submission record its full scientific and provenance value.

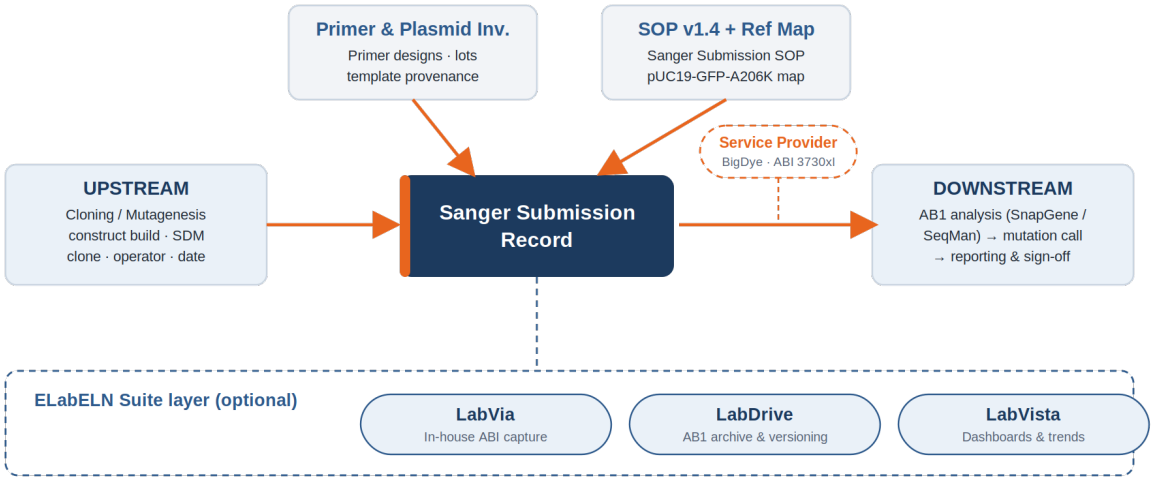

Figure 3. The template connects upstream cloning, parallel inventory and SOP/reference systems, an off-site sequencing service provider, and downstream AB1 analysis and reporting.

5.1 Upstream: Cloning and Mutagenesis

A Sanger submission verifies a construct that an earlier experiment created. The template links to the upstream cloning or site-directed-mutagenesis record, so opening the submission surfaces the construct's build history, the clone identifier, and the operator and date — making the verification traceable back to the molecule's origin without manual cross-referencing.

5.2 Parallel: Primer, Plasmid, and Provider Inventory

The Compounds section links the sequencing primer, the template plasmid, and submission reagents directly to the ELabELN inventory. Through the link the record automatically carries:

- **Primer designs and identifiers**, so the exact primer that produced a read is on the record.
- **Template lot and provenance**, tying the read to the specific plasmid stock submitted.
- **Low-stock warnings** before submission, so a batch is not prepared against an exhausted stock.

Service-provider records are maintained the same way, so submission metadata flows into every Sanger record without re-keying.

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5.3 Parallel: SOPs and the Reference Sequence Map

The Links section connects the submission to the lab's version-controlled Sanger Submission SOP (v1.4 by default) and to the reference sequence map (pUC19-GFP-A206K). The reference map is the fixed point every call is made against, and keeping it linked means each submission records exactly which reference and which SOP version it was assessed under.

5.4 Off-Site: The Sequencing Service Provider

Steps 5–7 — submission, the BigDye capillary run, and receipt of the AB1 trace — occur at the service provider. The template treats this hand-off as a first-class part of the record: the provider, chemistry, and instrument are captured as typed fields, the submission identity is logged, and the returned AB1 download is auto-logged to the audit trail. For labs running an in-house ABI 3730 or 3500, the same fields apply and the instrument run can be captured directly through the Suite's LabVia layer.

5.5 Downstream: AB1 Analysis, Calling, and Reporting

Trace analysis itself is performed in specialist tools — SnapGene or SeqMan Pro — where the AB1 is base-called, trimmed, and aligned to the reference. ELabELN is the system of record around that analysis: the AB1 file, the aligned-reference attachment, the quality metrics, and the final typed mutation call are stored and linked in the experiment, and the spreadsheet editor captures per-sample submission identity and per-position trace quality for cross-construct tracking. The result is a single record that holds the submission, the evidence, and the conclusion together, feeding reporting and sign-off.

5.6 The ELabELN Suite: Instruments and Dashboards

Everything above is available in ELabELN Standard. Labs on the ELabELN Suite gain three layers that connect to the same record: LabVia captures runs from an in-house ABI instrument directly; LabDrive adds metadata search, versioning, and role-based access across AB1 archives and other lab files; and LabVista builds dashboards and trends on the structured submission and mutation-call metadata. The same audit trail and data-integrity controls span all layers.

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6. How the Scientist Benefits

The template is built to deliver value at the bench and to the project record alike. The benefits compound with every submission a lab sends.

6.1 Start From a Tested Baseline, Not a Blank Submission

The scaffold — protocol, typed fields, primer and template links, reference map — is already built. The scientist edits a working configuration to match the construct and primer they actually verify, rather than rebuilding the submission record every batch.

6.2 Document by Doing

Checking off each of the 10 steps as it happens produces a contemporaneous, timestamped record as a by-product of running the submission. The AB1 download is logged automatically; there is no separate write-up to reconstruct later.

6.3 Keep Submission and Clone Provenance Intact

Because the primer, template, and provider are linked and the trace is attached, the exact inputs and evidence behind a confirmed clone are captured permanently. When a clone must be defended for a publication or patent months later, the submission, the AB1, and the call are all on one record.

6.4 Query Mutation Calls Across Submissions

Typed Mutation Calling fields make outcomes queryable. A scientist can pull every Sanger submission for a construct and see the full mutagenesis trail — intended versus observed, secondary peaks, alignment status — in seconds, instead of searching provider portals.

6.5 Produce Audit-Ready Records Without Extra Effort

The tamper-evident audit trail, version-controlled SOP and reference linkage, and electronic signatures mean the record that results from normal use is already structured to support reproducibility, clone-provenance documentation, and FDA 21 CFR Part 11 review when the lab's quality system requires it.

> **AT THE BENCH, IN PRACTICE**
> A graduate student opens the template, sets the construct and primer to match today's clones, prepares and quantifies the miniprep, submits to the provider and records the submission ID, and checks off each step. When the AB1 returns it attaches to the record automatically; the student aligns it in SnapGene, records Q30 and the observed mutation, and requests the project lead's electronic sign-off. The confirmed clone now has a complete, queryable provenance record — built with no documentation effort beyond doing the work.

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7. The Reference Verification and AB1 Trace

7.1 The Default Reference: A206K in pUC19-GFP

The template ships configured to verify the A206K monomerizing mutation in a pUC19-GFP construct — a recognizable, widely used edit that makes GFP monomeric. A single forward primer (pUC19-fwd) reads across the mutation site, and the call is made by aligning the returned trace to the pUC19-GFP-A206K reference map. This concrete reference demonstrates the full submission-and-analysis workflow out of the box; swap the construct, primer, or reference to match what your lab verifies.

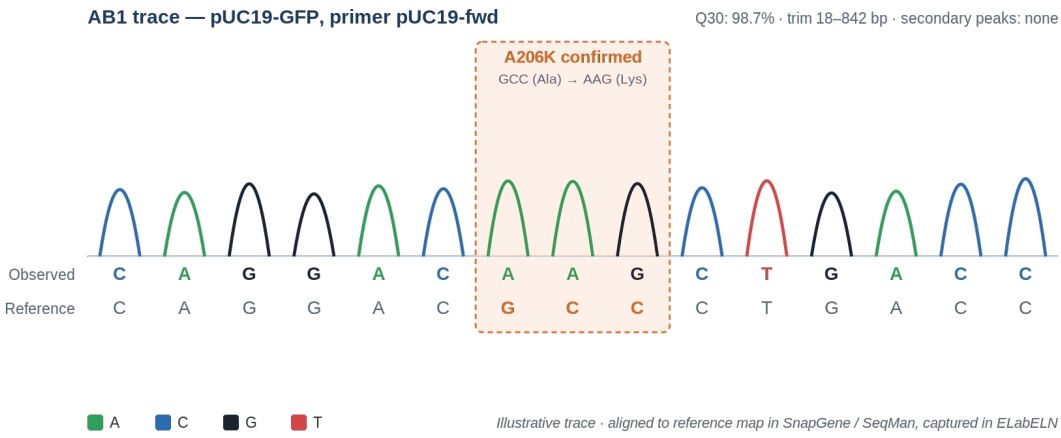
AB1 trace — pUC19-GFP, primer pUC19-fwd
Q30: 98.7% · trim 18–842 bp · secondary peaks: none
A206K confirmed
GCC (Ala) → AAG (Lys)
Observed C A G G A C A A G C T G A C C
Reference C A G G A C G C C C T G A C C
A C G T
Illustrative trace · aligned to reference map in SnapGene / SeqMan, captured in ELabELN

Figure 4. An AB1 chromatogram with the A206K codon confirmed against the reference — the observed clone reads the intended codon, with quality metrics captured alongside. Illustrative trace; alignment is performed in SnapGene / SeqMan and captured in ELabELN.

7.2 How the Template Handles Trace Quality and Calling

ELabELN captures the outcome of the analysis rather than performing the base-calling itself. The Quality fields hold read length, Q30 percentage, and trim positions; the Mutation Calling fields hold the intended mutation, the observed mutation, any secondary peaks, and the alignment status. The AB1 trace and the aligned reference attach to the same record, and the spreadsheet editor captures per-sample submission identity and per-position trace quality so verification can be tracked across constructs. Because the fields are typed, they are queryable across the construct's entire sequencing history.

Element	Default configuration	Editable to
Template type	Plasmid (pUC19-GFP)	PCR product, genomic DNA
Primer	Single forward (pUC19-fwd)	Custom primer; bidirectional; primer walking
Service provider	Eurofins / GENEWIZ	Macrogen, in-house ABI 3730 / 3500
Chemistry	BigDye Terminator v3.1	BigDye v3.0, v1.1
Reference call	A206K (intended vs observed)	Any defined edit or identity check

Figure 5. The template's default configuration — fully editable for any construct, primer, provider, or chemistry.

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8. Configuring the Template for Your Submission

Adapting the template to a different construct is a matter of editing the relevant sections — not rebuilding the structure.

8.1 Change the Template Type

Set the template type to plasmid, PCR product, or genomic DNA to match what you are verifying. The submission and quality fields apply across all three.

8.2 Swap the Primer, Provider, and Chemistry

Replace the primer with your standard cloning-vector primer, a custom primer, or a primer-walking set; change the service provider (Eurofins, GENEWIZ/Azenta, Macrogen, or an in-house ABI 3730/3500); and adjust the chemistry (BigDye v3.1, v3.0, or v1.1). If the primer and template already exist in the ELabELN compound database, simply re-link them.

8.3 Move to Bidirectional or Primer Walking

For longer inserts, edit the submission section to move from a single forward read to bidirectional sequencing or a primer-walking set, capturing each read against the same reference map.

8.4 Save a Custom Version

Once configured, save your edits as a private template scoped to your lab, or publish back to the ELabELN template library. Every subsequent submission of that construct opens from your configured version, and separate versions can be maintained for different constructs, providers, or regulatory contexts.

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9. Getting Started

ELabELN Standard is a cloud-hosted SaaS deployment typically live within one to two business days of account creation. There is no hardware to buy, no IT project, and no per-seat licensing.

1. **Start an ELabELN account.** Visit elabeln.com/get-started. ELabELN Standard runs on LabLynx's managed cloud infrastructure.
2. **Provision your instance.** LabLynx provisions the instance within one to two business days; you receive a URL, admin credentials, and onboarding documentation.
3. **Load the Sanger template.** From the template library, select the Sanger sequencing template and click “Use This Template.” An editable instance opens immediately.
4. **Add your primers, templates, and providers to the inventory.** Enter your sequencing primers, template plasmids, and service-provider records. This is a one-time setup step.
5. **Link your SOP and reference map.** Upload your Sanger submission SOP and the reference sequence map, and connect them to the template's Links section.
6. **Customize the template for your construct.** Edit the template type, primer, provider, and chemistry, then save as a private team version.
7. **Run your first submission.** Open your saved template, complete the 10-step checklist, attach the AB1 trace and aligned reference, and have the project lead sign off electronically.

> **TYPICAL TIMELINE**
> Day 1: Account requested at elabeln.com/get-started. Day 2–3: Instance provisioned; primer/template/provider inventory populated; SOP and reference map uploaded. Day 3–4: Template configured and saved as a private version for your construct. Day 4–5: First submission documented in ELabELN with audit trail and e-signature.

9.1 ELabELN Standard vs. ELabELN Suite

Capability	Standard	Suite
Sanger template access	Included	Included
Unlimited users	Included	Included
Audit trail and e-signatures	Included	Included
Primer / template / provider inventory	Included	Included
SOP & reference-map linking with version control	Included	Included
AB1 trace + aligned-reference attachments	Included	Included
LabVia direct in-house ABI capture	—	Suite layer
LabDrive AB1 archive metadata search & versioning	—	Suite layer
LabVista dashboards and trends	—	Suite layer

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10. Frequently Asked Questions

Can I configure the template for my construct and primer?

Yes. Every section is editable. Change the template type (plasmid, PCR amplicon, genomic DNA), swap the primer (standard cloning-vector primer, custom primer, or primer-walking set), switch the service provider (Eurofins, GENEWIZ/Azenta, Macrogen, or in-house ABI 3730/3500), adjust the chemistry (BigDye v3.1, v3.0, v1.1), or move from single-primer to bidirectional sequencing. Save your edits as a private template, or publish back to the library.

How does the template differ from the NGS templates?

Sanger reads 700–900 bp from a single primer with very high per-base accuracy — ideal for clone verification, mutagenesis confirmation, and small targeted reads. The NGS templates (RNA-Seq, scRNA-Seq, ChIP-Seq) read millions of short fragments in parallel, suited to quantitative expression profiling, variant calling at scale, or epigenomic profiling. Choose Sanger when you need an accurate read of a defined region; choose NGS when you need throughput.

Does the template track mutation calls across submissions?

Yes. The typed Mutation Calling group holds the intended mutation, observed mutation, secondary peaks, and alignment status, all queryable across a construct's sequencing history. Combined with the linked reference map, your lab can pull every Sanger submission for a construct and see the full mutagenesis trail — supporting clone-provenance documentation for publications and patent applications.

Does ELabELN analyze the AB1 trace itself?

No — base-calling and alignment are performed in specialist tools such as SnapGene or SeqMan Pro. ELabELN is the system of record around that analysis: it stores and links the AB1 file and the aligned reference, captures the quality metrics and the final mutation call as typed fields, and timestamps every step. This keeps the submission, the evidence, and the conclusion together in one auditable record.

Is it included in every ELabELN plan?

Yes. Every published template in the ELabELN library is available to all subscribers in both the Standard and Suite editions, with unlimited users in either edition and no per-template fee.

How quickly can my lab start running Sanger submissions in ELabELN?

ELabELN Standard cloud deployment is typically live in one to two business days. Once the instance is provisioned, the template loads from the library in a single click, with the construct, primer, service provider, and chemistry editable to match the submissions your lab actually sends.

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11. Conclusion

The ELabELN Sanger Sequencing Experiment Template is not simply a digital version of a submission form. It is a structured, audit-ready, searchable record that connects to your primer and plasmid inventory, your SOPs and reference maps, your upstream cloning experiments, an off-site sequencing provider, and — in the Suite edition — your in-house instruments and analytics dashboards. It turns a workflow that normally scatters across portals, inboxes, and notebooks into a single record that holds the submission, the AB1 evidence, and the final call together.

For a laboratory that verifies constructs regularly, the benefit compounds quickly. Every submission produces a record that future researchers can find and trust. Every primer and template is traceable. Every reference and SOP version is documented. Every step is timestamped, and every call is authenticated — so the most common Sanger failure, lost clone provenance, is closed by default.

The template is available to all ELabELN subscribers today. A new instance is live in one to two business days, and the first structured submission is typically complete within a week of account creation.

GET STARTED

Visit elabeln.com/get-started to request your ELabELN instance, or contact LabLynx at 866.522.5969 or info@lablynx.com. Load the Sanger template, configure it for your construct and primer, and run your next submission from a tested, audit-ready baseline.

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